

**WHAT IS CLAIMED IS:**

1. A pulse-multiline excitation apparatus for analyzing a sample containing one or more fluorescent species, comprising:  
  
one or more lasers configured to emit two or more excitation lines, each excitation line having a different wavelength;  
  
a timing circuit coupled to the one or more lasers and configured to generate the two or more excitation lines sequentially according to a timing program to produce time-correlated fluorescence emission signals from the sample;  
  
a non-dispersive detector positioned to collect the time-correlated fluorescence emission signals emanating from the sample; and  
  
an analyzer coupled to the detector and configured to associate the time-correlated fluorescence emission signals with the timing program to identify constituents of the sample.
2. The apparatus of claim 1, wherein the detector and the analyzer are integral.
3. The apparatus of claim 1, wherein the two or more excitation lines intersect at the sample.
4. The apparatus of claim 1, wherein the two or more excitation lines are configured so that the two or more excitation lines do not intersect in the sample.
5. The apparatus of claim 1, wherein the two or more excitation lines are configured so that the two or more excitation lines are coaxial.

6. The apparatus of claim 1, further comprising an assembly of one or more prisms in operative relation with the one or more lasers and configured to render radiation of the two or more excitation lines substantially colinear.
7. The apparatus of claim 1, further comprising at least four excitation lines having four excitation wavelengths.
8. The apparatus of claim 7, further comprising at least eight excitation lines having eight excitation wavelengths.
9. The apparatus of claim 8, further comprising at least sixteen excitation lines having sixteen excitation wavelengths.
10. The apparatus of claim 1, wherein said sample is comprised in at least one capillary.
11. The apparatus of claim 1, wherein said sample is comprised in at least 4 capillaries.
12. The apparatus of claim 11, wherein said sample is comprised in at least 8 capillaries.
13. The apparatus of claim 12, wherein said sample is comprised in at least 16 capillaries.
14. The apparatus of claim 13, wherein said sample is comprised in at least 48 capillaries.
15. The apparatus of claim 14, wherein said sample is comprised in at least 96 capillaries.
16. The apparatus of claim 15, wherein said sample is comprised in at least 384 capillaries.
17. The apparatus of claim 1, further comprising a sheath flow cuvette.
18. The apparatus of claim 1, wherein the timing program comprises a delay between the firing of each laser of between about 10 femtosecond and about 5 seconds.
19. The apparatus of claim 18, wherein the timing program comprises a delay between the firing of each laser of between about 1 millisecond and about 100 milliseconds.

20. The apparatus of claim 18, wherein the timing program comprises a delay between the firing of each laser of between about 50 ps and about 500 ps.
21. The apparatus of claim 1, wherein at least one or more of the excitation lines is pulsed.
22. The apparatus of claim 21, wherein said pulsed excitation line is controlled by TTL logic.
23. The apparatus of claim 21, wherein said pulsed excitation line is controlled by mechanical or electronic means.
24. The apparatus of claim 22, wherein said apparatus generates a sequence of discrete excitation lines that are time-correlated with the fluorescence emission signals from the sample.
25. The apparatus of claim 1, wherein at least one of the lasers comprises a diode laser.
26. The apparatus of claim 1, wherein at least one of the lasers comprises a semiconductor laser.
27. The apparatus of claim 1, wherein at least one of the lasers comprises a gas laser.
28. The apparatus of claim 1, wherein at least one of the lasers comprises a diode pumped solid state laser.
29. The apparatus of claim 28, wherein at least one of the solid state lasers comprises a Neodymium laser.
30. The apparatus of claim 1, further comprising a Raman shifter in operable relation with at least one laser beam.
31. The apparatus of claim 1, wherein the excitation wavelength provided by each laser is optically matched to the absorption wavelength of each fluorophore.

32. The apparatus of claim 1, wherein the detector comprises a charged couple device.
33. The apparatus of claim 1, wherein the detector comprises a photomultiplier tube.
34. The apparatus of claim 1, wherein the detector comprises a silicon avalanche photodiode.
35. The apparatus of claim 1, wherein the detector comprises a silicon PIN detector.
36. The apparatus of claim 1, wherein the footprint of said device is less than 4 ft' x 4 ft' x 2ft.
37. The apparatus of claim 36, wherein the footprint of said device is less than 1ft x 1ft x 2ft.
38. The apparatus of claim 36, wherein the footprint of said device is less than 1-in x 3-in x 6-in.
39. A method of identifying sample components comprising:
- (a) preparing a sample comprising sample components, a first dye and a second dye;
  - (b) placing the sample in the beam path of a first excitation line and a second excitation line;
  - (c) sequentially firing the first excitation line and the second excitation line;
  - (d) collecting fluorescence signals from the samples as a function of time; and
  - (e) sorting the fluorescence by each excitation line's on-time window,
- wherein the sample components are identified.
40. The method of claim 39, wherein the fluorescence signals are collected from discrete time periods in which no excitation line is incident on the sample, the time periods occurring between the firing of the two excitation lines.

41. The method of claim 39, wherein the absorption maxima of the first dye substantially corresponds to the excitation wavelength of the first excitation line.
42. The method of claim 39, wherein the absorption maxima of the second dye substantially corresponds to the excitation wavelength of the second excitation line.
43. The method of claim 42, further comprising a third and fourth dye and a third and fourth excitation line, wherein the absorption maxima of the third and fourth dyes substantially correspond to the excitation wavelength of the third and fourth excitation lines.
44. The method of claim 43, further comprising a fifth, sixth, seventh and eighth dye and a fifth, sixth, seventh and eighth excitation line, wherein the absorption maxima of the fifth, sixth, seventh and eighth dyes substantially correspond to the excitation wavelength of the fifth, sixth, seventh and eighth excitation lines.
45. The method of claim 44, further comprising a ninth, tenth, eleventh, twelfth, thirteenth, fourteenth, fifteenth, and sixteenth excitation line, wherein the absorption maxima of the ninth, tenth, eleventh, twelfth, thirteenth, fourteenth, fifteenth, and sixteenth dyes substantially correspond to the excitation wavelength of the ninth, tenth, eleventh, twelfth, thirteenth, fourteenth, fifteenth, and sixteenth excitation lines.
46. The method of claim 39, wherein at least one of said dyes is a xanthene, fluorescein, rhodamine, BODIPY, cyanine, coumarin, pyrene, phthalocyanine, phycobiliprotein, Alexa, or squaraine dye.
47. The method of claim 46, wherein at least one of said dyes is a BODIPY dye.
48. The method of claim 39, wherein said sample components enable the determination of SNPs.

49. The method of claim 48, wherein said method is for the high-throughput identification of informative SNPs.
50. The method of claim 48, wherein said SNPs are obtained directly from genomic DNA material.
51. The method of claim 48, wherein said SNPs are obtained from PCR amplified material.
52. The method of claim 48, wherein said SNPs are obtained from cloned material derived directly from genomic DNA material or PCR amplified material.
53. The method of claim 48, wherein said SNPs are obtained using a single nucleotide primer extension method.
54. The method of claim 50, wherein said single nucleotide primer extension method comprises using single unlabeled dNTPs, single labeled dNTPs, single 3'-modified dNTPs, single base-modified 3'-dNTPs, single alpha-thio-dNTPs or single labeled 2',3'-dideoxynucleotides
55. The method of claim 39, comprising a mini-sequencing method comprises using single unlabeled dNTPs, single labeled dNTPs, single 3'-modified dNTPs, single base-modified 3'-dNTPs, single alpha-thio-dNTPs or single labeled 2',3'-dideoxynucleotides.
56. The method of claim 55, wherein said mini-sequencing method comprises an SNP.
57. The method of claim 56, wherein said mini-sequencing method comprises multiple SNPs.
58. The method of claim 48, wherein said SNPs are obtained using Sanger sequencing.
59. The method of claim 48, wherein the analyzing of said signals is adapted for the accurate diagnosis of inherited disease, better prognosis of risk susceptibilities, identification of sporadic mutations, or prescribing tailor-made daily drug regimens for individual patients.

60. The method of claim 39, wherein the analyzing of said signals is adapted for routine usage in clinical diagnostics, forensics applications or determining general sequencing methodologies.

61. A method of identifying sample components comprising:

- (a) obtaining a biological sample;
- (b) labeling said sample with one or more fluorophores;
- (c) separating components of said sample; and
- (d) detecting said sample components with a device wherein said device comprises:
  - one or more lasers configured to emit two or more excitation lines, each excitation line having a different excitation wavelength;
  - a timing circuit coupled to the one or more lasers and configured to fire the two or more excitation lines sequentially according to a timing program to produce time-correlated fluorescence emission signals from the sample; and
  - a non-dispersive detector positioned to collect the time-correlated fluorescence emission signals;

wherein said detector collects time correlated data from said sample comprising fluorescent emissions of the sample as a result of irradiation by the one or more excitation lines.

62. The method of claim 61, wherein said sample components are nucleic acids.

63. The method of claim 61, wherein said sample components are amino acids.

64. The method of claim 61, wherein said sample components are proteins.

65. The method of claim 61, wherein said separating is by electrophoresis.
66. The method of claim 61, wherein said separating is by chromatography.
67. The method of claim 61, wherein said separating is by mass spectrometry.
68. The method of claim 61, wherein said sample components are addressed on high density chip arrays.
69. The method of claim 61, further comprising:
- (e) contacting said sample components on a surface comprising immobilized oligonucleotides at known locations on said surface; and
  - (f) performing a single nucleotide incorporation assay.
70. The method of claim 61, further comprising:
- (e) contacting said sample components on a surface comprising immobilized oligonucleotides at known locations on said surface; and
  - (f) performing a mini-sequencing assay.
71. The method of claim 69, further comprising rastering said surface or said excitation lines such that said excitation lines contact said surface at multiple locations.
72. A device comprising:
- (a) one or more lasers having two or more excitation lines;
  - (b) one or more beam steering mirrors wherein said excitation lines each strike said mirrors;



- (c) a first prism, wherein said two or more excitation lines strike one surface and exit from a second surface of said first prism; and
- (d) a second prism at an angle relative to said first prism, wherein said two or more excitation lines strike one surface of said second prism after exiting said first prism and exit said second prism,

wherein said two or more excitation lines are substantially colinear or coaxial after exiting said second prism.

73. The method of claim 72, wherein said two or more excitation lines are substantially coaxial after exiting said second prism.

74. The method of claim 72, wherein said two or more excitation lines are substantially colinear after exiting said second prism.

75. A method of illuminating a sample comprising:

- (a) steering two or more excitation lines onto a first surface of a first prism;
- (b) steering two or more excitation lines from the second surface of said first prism to a first surface of a second prism; wherein said second prism is angled about 45° from said first prism;
- (c) steering said two or more excitation lines onto a sample after exiting second surface of said second prism, wherein said two or more excitation lines are substantially colinear or coaxial after exiting said second prism.

76. The method of claim 75, wherein said two or more excitation lines are substantially coaxial after exiting said second prism.

77. The method of claim 75, wherein said two or more excitation lines are substantially colinear after exiting said second prism.

78. A method of controlling a sequence of excitation lines comprising:

obtaining a TTL circuit comprising an electronic stepper wherein said circuit is operationally connected to one or more lasers having two or more excitation lines; and

controlling the sequential firing of the one or more lasers having two or more excitation lines with a clock pulse from the circuit, wherein the frequency of firing one laser is equivalent to the frequency of firing a second laser, but phased shifted so that one or more lasers having two or more excitation lines can be sequentially pulsed.

79. The method of claim 78, wherein the cycle time of one clock pulse is from 1  $\mu$ second to 5 seconds.

80. The method of claim 78, wherein the length of time a first laser produces an excitation line is similar to the length of time a second laser produces an excitation line.

81. The method of claim 78, wherein between 2-to-16 excitation lines are sequentially pulsed.

82. The method of claim 81, wherein between 2-to-8 excitation lines are sequentially pulsed.

83. A method of controlling a sequence of excitation lines comprising:

obtaining a TTL circuit comprising an electronic stepper wherein said circuit is operationally connected to two or more lasers; and

controlling the sequential firing of the two or more lasers with a clock pulse from the circuit, wherein the frequency of firing a first laser is different from the frequency of firing a second laser.

84. The method of claim 83, comprising between 2-to-16 lasers.

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